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Validation of DNA-based HLA-A and HLA-B testing of volunteers for a bone marrow registry through parallel testing with serology

Key words:

serology; DNA typing; HLA-A; HLA-B; bone marrow registry

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Abstract: A total of 42,160 individuals were typed for HLA-A and HLA-B by both serology and PCR-based typing. The HLA assignments included all of the known serological equivalents. The majority of the individuals (99.9%) were from U.S. minority population groups. The serologic typing was performed between 1993 and 1997 at the time of recruitment for the National Bone Marrow Program (NMDP) registry. The polymerase chain reaction (PCR)-based typing was carried out in two phases. In phase I, DNA typing was performed by PCR using sequence-specific oligonucleotide probes (PCR-SSOP) or PCR using sequence-specific primers (PCR-SSP) without knowledge of the serologic assignments. Discrepancies were identified between the serologic and DNA assignments in 24% of the volunteers (8% of volunteers differed for only HLA-A assignments, 13% for HLA-B, and 3% for both HLA-A and -B) and a potential explanation was assigned each discrepant serology/DNA pair. In phase II, a random sampling scheme was used to select a statistically significant number of individuals for repeat DNA typing from each of these categories. The categories included antigens missed by serology, nonexpressed (null) alleles, PCR amplification failures, misassignment of antigens and nomenclature issues. Only a single individual was found to carry a null allele. DNA-based testing correctly typed nearly 99% of the donors at HLA-A, more than 98% at HLA-B, and more than 97% at both HLA-A and -B validating this methodology for registry typing.

The National Marrow Donor Program (NMDP) registry lists approximately 4 million volunteer donors typed for HLA-A, B by serology. Several technical issues including reliability of serologic typing, the need for viable cells, and the limited supply of serologic reagents required to define all WHO-assigned specificities led the NMDP to begin to replace serologic HLA-A, B typing by DNA-based typing. An additional limitation of serologic typing was the high discrepancy rates between serologic and DNA-based typing observed in non-Caucasian populations in other studies. Opelz and colleagues (1), for example, reported a 36% discrepancy rate in the serologic assignments as compared to DNA-based typing of individ-

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uals of direct African ancestry in the Collaborative Transplant Study.

The primary goal of this study was to validate DNA-based HLA-A and HLA-B testing of volunteer donors for the NMDP registry by comparison of the DNA results with the serologic assignments obtained from the same individuals. This study complements a recent report which evaluates the accuracy of DNA-based HLA assignments of blinded quality control samples tested during registry typing (2). A second goal was to identify HLA-A and -B serologic assignments which might be reported incorrectly during registry typing to aid in the design of search strategies for identification of matched hematopoietic stem cell donors. A third goal was to determine whether known non-expressed (null) HLA-A and B alleles should be identified in the registry typing strategy, that is, whether these alleles are frequently encountered in typing of US populations.

Material and methods

A total of 42,160 volunteer donors for the NMDP registry serologically typed during the period 1993–1997 were selected for the study. Selection for DNA testing in phase I was based on several factors. The volunteer donor must have had a frozen whole blood sample stored in an NMDP repository and HLA-DR typing must have been reported on the volunteer. Because serologic HLA typing on individuals from minority populations was technically more difficult due to lack of specific alloantisera, samples from minority volunteer donors were prioritized for re-typing. Ninety-nine percent of the samples tested were from US minority populations including: 41% African American, 31% Hispanic, 21% Asian / Pacific Islander, 4% Native American, 2% mixed background and 1% other. Specimens included in this analysis were limited to those tested by phase I DNA laboratories that met the minimum criteria for data quality ($\leq 4\%$ error rate) as monitored by blinded quality control testing (2).

The volunteer donors were serologically typed for HLA-A and HLA-B at the time of recruitment using standard complement-dependent cytotoxicity techniques. NMDP Donor Centers selected the serologic typing laboratories. The identities of the typing laboratories were not collected by the NMDP and donor lymphocytes were not available for repeat typing by serology.

In phase I, DNA-based typing of the 42,160 volunteers was performed in 14 NMDP-affiliated laboratories between June 1997 and March 1998. The laboratories did not know the serologic assignments prior to typing. DNA was extracted from frozen whole blood specimens by various methods. Thirteen laboratories performed

PCR-SSOP typing using a set of approximately 30 HLA-A locus probes and approximately 47 HLA-B locus probes. Although the probes were not standardized for all labs, 9 labs used a common set of probes from a single vendor (Lifecodes, Stamford, CT, USA) and 4 labs used a customized set of probes. Some laboratories used supplemental probes, additional group specific amplification, or SSP to achieve the required resolution. One laboratory performed SSP using 24 HLA-A locus and 48 HLA-B locus primer pairs. The resolution of the typing was defined as "serologic split" level (antigens/alleles are listed in Table 3) with the exception of alleles in the B*14, B*15 and B*40 groups. Typings which identified B*15 alleles were required to resolve the assignments into one of two groups which approximately corresponded to the B15 (gr.1) and B70 (gr. 2) serologic assignments. B*14 and B*40 were not subdivided. Occasionally, broad types were assigned by serology or by DNA typing (e.g., A28 or A*28). Additional details of the typing methodologies, the resolution, and the quality of the typing during this time period have been reported (2).

Typings, both serologic and DNA-based, were submitted to the NMDP and the assignments were compared to one another. Discrepant assignments in which the typings did not fall into the same numerical antigen/allele group were flagged (e.g., A1 and A*01 were considered consistent assignments while B35 and B*53 were labeled discrepant). Typings in which only a single assignment at a locus was obtained were considered homozygous for the purpose of this study (e.g., A2 was considered A2,A2). In 0.26% of samples, the DNA typing laboratory failed to achieve amplification of the locus (i.e., neither allele amplified) and these samples were not included in the analysis. Since the goal of the study was to validate DNA-based typing using serology as the benchmark, the emphasis was on discrepancies obtained when the DNA-based procedures were performed correctly. Thus, discrepant results were sent back to the DNA testing laboratory for review and correction of clerical errors and/or misinterpretation of raw data. A total of 526 samples (1.2%) were attributed to these errors and corrected DNA results were re-submitted to NMDP. It was not possible for the serologic laboratories to review the phase I discrepancies because the typings were performed over a period of five years and by numerous unidentified laboratories. Clerical errors may have been more frequent in the serologic assignments submitted to the registry because these laboratories typically reported their results to an NMDP donor center and the donor center reported the results electronically to the NMDP. Typings that remained discrepant following the review of DNA-based results were further evaluated in phase II.

In phase II, typings were subdivided into categories based on the type of discrepancy and the predicted explanation for the discrepancy (Table 1). To facilitate the selection of samples for repeat

Types of discrepancies observed

Group	Serology	DNA	Examples	Potential explanation	Number	Retest	Resolution of DNA re-test ^a
Group 1	Homozygous	Heterozygous	A2 vs. A*02,*33	Serology blank	2876	115	Serology level
Group 2	Homozygous	Heterozygous	A2 vs. A*02,*03	Null allele	170	147 ^{b,c}	Allele level
Group 3	Heterozygous	Homozygous	A1,2 vs. A*01	Amplification failure (DNA blank) or double dose effect	637	49	Serology level
Group 4	Homozygous or heterozygous	Homozygous or heterozygous	A23,31 vs. A*23,*30	Misassigned antigen/allele	4385	267	Serology level
Group 5	Mixed categories of discrepancies	Mixed categories of discrepancies	See text	Blanks and misassigned at A and B loci or incorrect sample tested	1613	72	Serology level
Total					9681/ 42160	650/ 9681	

^a Potential nomenclature discrepancies are in all categories and were typed at allele level

^b 22 samples were unavailable for retesting

^c 2 samples were found in other groups

Table 1

typing, each sample was assigned to a single category even though it may have had more than one type of discrepancy. To estimate how often the DNA typing was correct, a statistical approach was designed to randomly select individuals from these categories for retesting. A stratified random sample of 672 cases was selected. The number of samples selected from each group varied because the groups differed in how much information they were anticipated to yield. Some groups were intentionally over- or under-represented in the sampling in an attempt to maximize the amount of statistical information. For example, potential null alleles were over-represented since little was known about the frequency of nonexpressed alleles in the US population. Categories with well-known serologic limitations were under-sampled. Statistical weighting of the results

was performed to adjust for this sampling (3). Cases where the DNA and serologic typings were not discrepant were assumed to be correct and not considered for retesting.

In phase II of the study, one laboratory was contracted to repeat the DNA-based typing of the 672 selected samples at either a serologic equivalent or allele level resolution using SSOP, SSP and/or sequencing based typing (SBT). Twenty-two of the samples were unavailable for retesting. The level of resolution chosen for each sample depended on the category of the proposed discrepancy and the type of discrepancy. For example, samples that represented potential null alleles or nomenclature issues were typed at allele level while samples that included potential serologic blanks or amplification failures were tested at a lower resolution. Samples with po-

Null alleles

Table 2

Null allele	Closely related allele	Sequence mismatch location ^a	Number tested in broad allele category	Results (null allele positive)
A*0105N	A*0101	Exon 4: 131 C replaces GG	3	0
A*0215N	A*0207	Exon 4: 224 A replaces C	16	0
A*0303N	A*03011	Exon 3: 30-35 Deletion	23	0
A*2409N	A*2402	Exon 4:123 T replaces C	55	1
A*2411N		Exon 4: 8 CC replaces C		
A*2611N	A*2601	Exon 3: 173 GAC replaces G	2	0
B*1526N	B*15011	Exon1:11 T replaces C	39	0
		Exon 3: 26 A replaces C		
B*5111N	B*51011	Exon 4: 8 CA replaces A	12	0

^a Exon and nucleotide position

Correlation of Serologic and DNA Assignments

Table 3

Serologic assignment	N	% DNA assignments consistent with serology	DNA assignment	N	% serology assignments consistent with DNA
A1	5976	95	01XX	5716	99
A2	20146	97	02XX	19713	99
A3	6604	97	03XX	6482	99
A9	6	100	09XX	100	90
A10	35	91	10XX	266	92
A11	5317	98	11XX	5313	98
A19	42	83	19XX	3	33
A23	4776	96	23XX	4728	96
A24	8894	97	24XX	8831	98
A25	516	95	25XX	508	96
A26	2684	78	26XX	2045	96
A28	5987	96	28XX	306	91
A29	2803	95	29XX	2709	98
A30	6757	93	30XX	6543	96
A31	2592	90	31XX	2615	89
A32	1626	94	32XX	1559	98
A33	5179	92	33XX	5152	93
A34	1629	75	34XX	1278	90
A36	747	92	36XX	938	74
A43	0	0	43XX	5	0
A66	177	79	66XX	889	16
A68	684	90	68XX	6213	96
A69	38	37	69XX	131	85
A74	1081	90	74XX	2008	50
A80	16	100	80XX	261	6
B5	33	55	05XX	211	98
B7	6870	87	07XX	6040	99
B8	3195	96	08XX	3113	99
B12	5	60			
B13	1718	97	13XX	1696	97
B14 ^a	2667	97	14XX ^a	2809	98
B15 ^a	61	83	15(gr1) ^a	5181	93
B16	17	94	16XX	62	97
B17	67	93	17XX	1	100
B18	2712	95	18XX	2711	95
B21	12	33	21XX	47	98
B22	43	49	22XX	81	89
B27	1749	98	27XX	1746	98
B35	8974	90	35XX	8461	95
B37	744	96	37XX	748	95
B38	1280	95	38XX	1232	94

Table 3 continued

Serologic assignment	N	% DNA assignments consistent with serology	DNA assignment	N	% serology assignments consistent with DNA
B39	2692	94	39XX	2680	95
B40 ^a	64	84	40XX ^a	5830	94
B41	752	92	41XX	764	91
B42	2092	95	42XX	2128	94
B44	7088	97	44XX	6966	99
B45	2297	93	45XX	2212	97
B46	742	94	46XX	935	75
B47	107	79	47XX	111	77
B48	916	90	48XX	1077	77
B49	1714	96	49XX	1683	96
B50	961	82	50XX	903	86
B51	4384	90	51XX	4037	94
B52	1993	91	52XX	2029	88
B53	4447	93	53XX	4540	91
B54	391	94	54XX	534	70
B55	958	85	55XX	857	91
B56	390	81	56XX	330	88
B57	3809	72	57XX	2886	96
B58	3167	95	58XX	4097	74
B59	59	86	59XX	94	54
B60 ^a	2858	91			
B61 ^a	2930	96			
B62 ^a	3761	91			
B63 ^a	1063	94			
B64 ^a	52	96			
B65 ^a	123	95			
B67	37	65	67XX	113	21
B70 ^a	3570	92	15(g2) ^a	4151	82
B71 ^a	33	82			
B72 ^a	95	94			
B73	12	17	73XX	5	40
B75 ^a	334	93			
B76 ^a	22	84			
B77 ^a	20	78			
B78	139	86	78XX	390	31
B81	20	75	81XX	637	3
B82	0	0	82XX	99	0

^a Splits not resolved by DNA: B40 (60,61), B14 (64,65), B15 gr1 (62,63,75,76,77), B15 gr2 (70,71,72)

tential PCR amplification failures were tested using different pairs of amplification primers. The null alleles evaluated (Table 2) included: A*0105N, A*0215N, A*0303N, A*2409N, A*2411N, A*2611N, B*1526N, and B*5111N (4–9).

Results and discussion

Of the 42,160 individuals typed for HLA-A and HLA-B by both serology and PCR-based testing, 24% or 9,681 individuals had typings which remained discrepant following review of the DNA assignments by the DNA typing laboratory. The majority of discrepancies (13%) were HLA-B assignments alone; 8% of individuals carried a discrepant HLA-A assignment and 3% of individuals had discrepancies at both HLA-A and HLA-B. A single discrepancy included either a single difference at a locus (e.g., A*23, A*66 compared to A23, A34) or differences in both assignments at a locus (e.g., A*33 compared to A28, A34).

The overall correlation between specific serologic and DNA assignments for the 42,160 individuals typed in phase I are shown in Table 3. At the serologic level, all antigens except A43 and B82 were observed. At the resolution used in the DNA typing, all allele groups (e.g., A*01) known at the time of the testing were observed. For HLA-A, the percent of DNA assignments consistent with serology varied from 100% to 37%. For HLA-B, the consistency in assignments ranged from 98% to 17%. Table 3 also lists the percent of serologic assignments consistent with the DNA typing results. For HLA-A, these values varied from 99% to 0% and, for HLA-B, from 99% to 0%. For example, of the 1,081 serologic A74 assignments, 90% were typed as A*74 by DNA. In contrast, of the 2,008 samples identified as carrying the A*74 allele, only 50% were assigned as A74 by serology. The failure to detect A74 was likely due to limitations in the serologic reagents defining this antigen. The low values (<90%) for HLA-A26, 31, 34, 36, 43, 66, 69, 74, and 80 are similar to results observed in the UCLA Cell Exchange (10). However, the exchange did not list A43; A26 was considered well defined and A68, a developing antigen. The low values for HLA-B7, 15, 40, 46, 47, 48, 50, 52, 54, 55, 56, 57, 58, 59, 67, 70, 73, 76, 77, 78, 81, 82 reflect data on the difficulties of detecting some of these antigens. Differences from the UCLA study are the presence of B7 (considered well defined) and B55 (not listed in the UCLA study), and the absence of B49, 53 (considered developing). For B7, the inconsistency results from the misidentification of B*81 as B7. Overall, the discrepancies observed were not unexpected and include known limitations in serologic testing.

The discrepancies were categorized into five groups based on

the type of discrepancy and the proposed explanation of the discrepancy (Table 1). Groups 1 and 2 included the samples thought to have antigens missed by serology but identified by DNA typing (serology homozygous and DNA heterozygous). Group 2 included a subset of serologic missed antigens that were potentially non-expressed (null) alleles. Group 3 included samples homozygous for DNA and heterozygous for serology which were considered potential PCR amplification failures. Group 4 included samples in which serology and DNA did not match (i.e., potentially misassigned). Group 5 included samples with multiple discrepancies and included a mix of both blank and misassigned antigens. To facilitate subsequent retesting, each sample was placed in a single category for sampling. A sampling scheme was used to select the 650 samples which were retested using DNA-based methods.

Typings in which serology detected only a single HLA antigen while DNA typing detected two allele groups represented 31.4% of the overall phase I discrepant samples (Table 1, Groups 1, 29.7% and 2, 1.7%). There were several potential explanations for the serologic "blanks" including: 1) Lack of reagents to define new antigens; 2) Lack of monospecific reagents; 3) Presence of an antigen which masked a second assignment; and 4) Non-expressed (null) alleles. The antigens most frequently missed by serology were: A74, 33, 80, 68, 31, 30 and B15gr2 (70), 46, 58, 53, 40, 35 (in order of frequency).

It is well known that some HLA-A and -B assignments were (and are) difficult to define by serology. These include antigens which were characterized recently and for which alloantisera were not available at the time of the serologic testing. An example is A80 found as A*80 by DNA in 261 individuals in phase I, but by serology in only 16 individuals. Although A8001 was officially assigned as an HLA-A specificity by the WHO Nomenclature Committee in 1992, it was not described in a workshop until 1996 and was not widely identified by serologic typing until after that workshop (11). Phase II retesting of eight of the 246 individuals with discrepant typing involving A*80 found that 100% were consistent with the original DNA typing and discrepant with the serology assignment. Five out of the eight samples were serologically typed before 1996. Likewise B81 and B82 were not detected at the serologic level due to the lack of reagents and their recent characterization.

Undetected serologic antigens also occurred frequently for specificities which are difficult to define either due to lack of monospecific alloantisera (such as A34 and A66) or because of antigen combinations that mask the second assignment due to cross-reactivity (e.g., A*33,*74-positive cells typed as A33 or B*51,B*52-positive cells typed as B51). In phase II of this study, 114 samples were retested out of the 2,876 in Group 1, 100% were consistent with the DNA-based assignment rather than the serologic type.

Potential null or non-expressed alleles may also give rise to

homozygous serologic assignments compared to heterozygous DNA assignments. This study focused on null alleles which had been described in the literature most of which were studies of caucasoid populations (Table 2). The exceptions were A*0215N and B*1526N which had been originally described in the Japanese population. All of the discrepant samples (Table 1, Group 2) in which the serologic blank corresponded to a DNA low-resolution assignment that included a known null allele (e.g., A2 vs A*02, A*03 where the A*03 allele present may be A*0303N) were re-typed in phase II using allele level resolution to test for the presence of the null allele. Only 1 of 99 individuals potentially carrying HLA-A locus non-expressed alleles was found to be positive for a null allele (A*2409N) and none of the 50 individuals carrying potential HLA-B null alleles were positive for a known non-expressed allele (2 samples were found in groups other than Group 2). While it is possible that other serologic blanks placed in Group 1 resulted from new currently undefined non-expressed alleles, review of the data suggested that it is more likely that the reason for the failure of serology to detect the antigen was due to the known limitations of serology as discussed in the previous section. It should be noted that essentially all of the individuals tested in this study were from US minority populations; the presence of non-expressed alleles found solely in US Caucasoid populations was not evaluated.

Cases where DNA-based typing was homozygous compared to heterozygous by serology represented 6.6% of the phase I discrepant samples (Table 1, Group 3). One likely explanation for these discrepancies was a failure to achieve PCR amplification of one of the two alleles. Of the 637 phase I discrepant samples in this category, 49 were selected for retesting by DNA using alternative primer pairs in the phase II laboratory. Amplification failures occurred in 22% of the 49 discrepant samples and included multiple examples of A*29, B*51, B*44 and B*73. These amplification failures occurred in the early stages of the Class I registry typing project when methods were not optimized (2). Improvement in typing protocols and primer design coupled with routine retesting of apparent homozygotes likely has reduced the number of amplification failures in later typings. Most of the samples (70%) were confirmed to be correctly assigned by DNA typing. The explanation for many of these discrepancies in Group 3 was that serologic homozygotes often give false positives with alloantisera detecting cross-reactive antigens due to a double dose of the antigen (e.g. A2, 28 vs A*02, *02). The discrepancies in the remaining 8% of phase II samples in this category were either unexplainable because no consensus was reached between the three typings or were discrepant due to nomenclature (discussed below).

Misassigned antigens formed the largest group of discrepancies from phase I including 45.4% of the phase I discrepant samples

Resolution of discrepancies

Type of result	A locus percent ^a	B locus percent ^a	A and B loci percent ^a
DNA matches serology	89.65±0.29	84.68±0.34	77.04±0.40
DNA correct ^b	99.21±0.09	98.61±0.11	97.91±0.14
DNA incorrect ^c	0.46±0.06	1.07±0.10	1.52±0.12
Serology incorrect ^{b,c,d}	9.29±0.28	13.67±0.33	20.60±0.39
Samples switched ^e	0.27±0.05	0.27±0.05	0.27±0.05
Null allele present	0.003±0.01	0.0	0.003±0.005
Unresolved ^f	0.24±0.05	0.20±0.04	0.44±0.06
Unable to repeat DNA typing ^g	0.10±0.03	0.13±0.04	0.13±0.04

^a 95% confidence interval

^b Apparent discrepancies caused by nomenclature were considered as correct DNA assignment and incorrect serology assignment for this analysis

^c DNA results on 526 samples (1.2%) were attributed to clerical or technical errors, corrected and re-submitted to NMDP

^d Serologic typing laboratories were not able to review the phase I discrepant results so that some of these errors may have resulted from clerical mistakes or misinterpretation of results

^e Samples in which it appears that the serology and DNA laboratories tested different samples. It is possible that other discrepancies with more subtle mismatches resulted from this problem but it was not possible to evaluate this in the study

^f Unresolved samples are those samples in which the phase II retesting identified a new assignment that did not correspond to either the serologic or phase II DNA assignment

^g 22 samples of the 672 were not available for retesting

Table 4

(Table 1, Group 4). A likely explanation for these discrepancies was the cross-reactive nature of the HLA antigens and the use of alloantisera which include multiple cross-reactive specificities (e.g. A23, 26 vs. A*23, *66 or B57, 35 vs. B*58, *35). The most frequently misassigned types were: A26, 34, 33, 31, 1, 30, 74 and B57, 7, 35, 70, 53, 50, 55 (in order of frequency). A*66 was most often misassigned by serology as A26 or A34; A*36 as A1; A*74 as A33; A*31, *30 as A30 or 31; B*58 as B57; and B*81 as B7. In this sample group, 267 out of 9681 samples were selected for repeat typing in phase II. Of these samples, 86% confirmed the original DNA typing, 8% confirmed the serology, and 6% were unexplainable or represented nomenclature differences (discussed below).

In Group 5 (Table 1) which represented the remaining 16.6% of discrepant samples, the samples had either multiple types of discrepancies (e.g., blank and misassigned) at one locus or discrepancies at both A and B loci. In some instances, it appeared that the serology and DNA typing laboratories tested different individuals in cases where discrepancies at both HLA-A and HLA-B were observed (e.g., A11, 33, B8, 44 vs A*24, B*52, *54). The remaining samples in Group 5 had the same categories of discrepancies as samples in Groups 1-4.

Apparent discrepancies were observed related to the nomenclature used in allele assignment. These complications have been previously reported and were attributed to the assignment of allele

names based on sequence homology and not based on serologic reactivity of the resultant molecule (12, 13). These included B*1522 encoding a protein receiving a serologic B35 assignment, B*2708 and B7, B*4005 and B50, and B*5002 and B45. In phase I, 893 of the 42,160 (2%) samples were identified as having discrepancies likely due to nomenclature. In phase II, 17 out of 893 samples were identified for repeat typing at high resolution. These samples were distributed among all five groups (Table 1). Upon retesting, 16 of the 17 samples were discrepant due to differences in nomenclature. It should be noted that, although nomenclature differences were counted as discrepant in this analysis, neither DNA nor serology should be considered as incorrect.

The phase II retesting identified a new assignment that did not correspond to either the serologic or phase I DNA assignment in 15 of the 650 samples undergoing repeat testing. In several cases, the phase II assignment identified a more recently defined allele which might have been missed in the initial DNA typing interpretation (e.g., serology B-blank vs. *15gr1 and the final typing by SBT was B*3528 where B*3528 was not known at the time of either the Phase I or II typing). In other cases, the phase II study identified an allele

closely related to the phase I or serologic assignments (e.g., A11, 26 vs. A*11, *34 vs. A*11, *66).

Evaluation of the typing results indicated that DNA based testing correctly typed nearly 99% of the donors at HLA-A, more than 98% at HLA-B, and more than 97% at both HLA-A and -B. The distribution of the results is shown in Table 4. Because the HLA assignments covered the entire spectrum of allele groups known at the time of the testing, this study provides a comprehensive analysis of the ability of DNA based typing to define all low resolution types and validates the transition of registry HLA-A and HLA-B typing protocols to DNA-based approaches. In addition, the study confirms previous observations on specific HLA antigens or antigen combinations that are likely to be missed or misassigned by serology as well as nomenclature issues which might confuse individuals responsible for HLA-matched donor selection. The results detected amplification failures and resulted in improvements in testing methods and procedures to reduce those errors. Finally, the study suggests that previously defined non-expressed alleles will be infrequent in US minority populations.

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